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# **Synthetically modified bioisosteres of salicyl alcohol and their gastroulcero-genic assessment versus aspirin: biochemical and histological correlates**

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## Abstract

The present study was conducted to synthesize nitrogen containing derivatives of salicyl alcohol and to investigate *in vivo* their ulcerogenic potential in comparison with aspirin in rats. The compounds [4-(2-hydroxybenzyl) morpholin-4-iumchloride (I)] and [1,4-bis (2-hydroxybenzyl) piperazine-1,4-dium chloride (II)] were synthesized and their chemical structures were characterized using spectral data. In our previous study (Ali et al., Afr J Pharm Pharmacol 7, 585-596, 2013) both compounds showed anti-inflammatory, antinociceptive and antipyretic properties in standard animal models and a greater binding affinity for cyclooxygenase-2 vs. cyclooxygenase-1 in molecular docking and dynamics analysis. For *in vivo* studies, animals were randomly divided into four groups. The synthetic compounds (both at 100 or 150 mg/kg), aspirin (150 mg/kg) or saline vehicle were administered orally, once daily for six days and then tested for ulcerogenic activity. At the end of the procedure, gastric juice and tissues were collected and subjected to biochemical and histological analysis. The results of the study revealed that in the case of the aspirin treated group, there was a significant increase in gastric juice volume, free acidity, total acidity, ulcer score and a decrease in gastric pH. Moreover, histological examination of the gastric mucosa of the aspirin treated group indicated morphological changes while neither of the synthetic compounds showed any significant ulcerogenic or cytotoxic properties. The results of the present study suggest that both compounds are free from ulcerogenic side effects and may represent a better alternative to aspirin.

**Keywords** Bioisosteric synthesis. [4-(2-hydroxybenzyl) morpholin-4-ium chloride]. [1,4-bis (2-hydroxybenzyl) piperazine-1,4-dium chloride]. Acetylsalicylic acid. Ulcerogenicity. Histology

## **Introduction**

Studies on the pharmacological mechanism(s) underlying nonsteroidal anti-inflammatory drugs (NSAIDs) have established an inhibitory link with cyclooxygenase (COX) enzyme activity (Amir and Shikha, 2004; Smith et al., 1998). There are three isoforms of the COX enzyme (Dannhardt and Kiefer, 2001; Marnett and Kalgutkar, 1999) namely COX-1, COX-2 and COX-3; the latter is a splice variant of COX-1 not thought to be expressed in humans (Warner and Mitchell, 2004).

Pain, inflammation and pyrexia frequently occur in combination, implicating common mediating pathways. So, it is not surprising that inhibitors of COX enzymes are used to treat these clinical disorders. COX-1 has a housekeeping or gastroprotective function while COX-2 is inducible and is predominantly involved in inflammation, pyrexia and pain (Almansa et al., 2003; Habeeb et al., 2001). The majority of the NSAIDs either inhibit all forms of COX enzyme non-selectively (Tapiero et al., 2002) or have greater affinity for COX-1 compared to COX-2 (Jackson and Hawkey, 1999). This is thought to be the origin of serious adverse effects such as peptic ulcer, perforation and gastrointestinal bleeding (Bulbena et al., 1993; Paulus, 1988; Singh and Rosen, 1998) associated with the use of NSAIDs and this imposes a major limitation of their therapeutic application (Tammara et al., 1994).

Currently, peptic ulcer is a major global health problem and the search for novel plant-derived or synthetic NSAID molecules devoid of ulcerogenic propensity, is an active area of investigation for biomedical researchers (Tammara et al., 1994). Certain biochemical actions of drugs are clinically advantageous in the diagnosis or treatment of disorders, yet others may be detrimental giving rise to adverse effects. Hence, alterations in chemical structure are necessary to enhance beneficial properties.

A range of biochemical activities have been reported for glycosides and it is principally the genin (aglycone) moiety which is responsible for these actions (Robyt, 1998; Salga et al., 2011; Yoon

et al., 2004). Salicin (2-(hydroxymethyl) phenyl- $\beta$ -D-glucopyranoside) is a glycoside chemically related to aspirin which may be hydrolyzed to yield salicyl alcohol (2-hydroxy methyl phenol or saligenin) (Table 1). Salicin has been reported to be an anti-rheumatic, anti-inflammatory and antipyretic pro-drug with no propensity to induce gastric damage (Akao et al., 2002; Yoon et al., 2004). These properties of salicin which is devoid of a  $\text{-COOH}$  group prompted the bioisosteric synthesis of new compounds lacking this group with a targeted pharmacological profile. Evidence regarding anesthetic as well as antipyretic activities of salicin have been reported (Charles and Tony, 1956; Machagan, 1876). The compounds under current study are bioisosteres derived from salicyl alcohol, via incorporation of pharmacologically important morpholine and piperazine moieties. Other compounds comprising such moieties inhibit eicosanoid pathways and exhibit varied pharmacological properties including antiproliferative activities (Berardi et al., 2008; Coonrod et al., 2001; Nozawa et al., 2007). In this regard, the piperazine moiety has been proposed as a critical core for novel drug design (Bali et al., 2010) and morpholine substitution in heterocyclics has yielded both anti-inflammatory and antinociceptive properties (Panneerselvam et al., 2009).

Bearing in mind the concept of salicin as a prodrug and the potential for pharmacological properties of compounds with morpholine or piperazine substitution, we performed the bioisosteric synthesis of compounds I [4-(2-hydroxybenzyl) morpholin-4-iumchloride] and II [1,4-bis(2-hydroxybenzyl)piperazine-1,4-diiumchloride] (Table 1, Fig. 1) and evaluated their ulcerogenic propensity.

In light of this, it is noteworthy that we have recently reported these compounds to possess significant anti-nociceptive, anti-inflammatory and anti-pyretic activities comparable to aspirin in animal models which were supported by molecular docking and dynamics simulation studies (Ali et al., 2013). Consequently, we now wished to establish the gastrointestinal tolerability of these compounds in comparison with aspirin.

## Methods and materials

### Chemicals

Bovine serum albumin and acetylsalicylic acid were obtained from Sigma. The starting materials were either purchased from Aldrich and Sigma or prepared by standard procedures available in the literature. All other chemicals used were of analytical grade and acquired commercially from local sources.

## Chemistry

### General

Infrared spectra (IR) were determined on a Nicolet 380 thermoscientific FTIR. Mass spectra were recorded on a JEOL MSRoute spectrometer and nuclear magnetic resonance (NMR) spectra scanned on a BRUKER 300-MHz spectrophotometer using D<sub>2</sub>O as a solvent. Melting points (mp) of compounds were determined using a Gallenkamp Melting Point apparatus. The chemical shifts were presented as parts per million ( $\delta$  ppm) using tetramethylsilane (TMS) as an internal standard.

## Synthesis

Compound I [4-(2-hydroxybenzyl)morpholin-4-ium chloride] and compound II [1,4-bis(2-hydroxybenzyl)piperazine-1,4-diium chloride] were synthesized (Fig. 1) using salicylaldehyde as a starting material according to the following synthetic protocol. A mixture of NaBH<sub>4</sub> (10 mmol), salicylaldehyde (10 mmol), and boric acid (10 mmol) were milled with mortar and pestle until thin layer chromatography exhibited entire vanishing of the starting material. The reaction mixture was quenched with a 10 ml solution of 1N HCl and extracted with ethyl acetate. The ethyl acetate layer was separated and dried over sodium sulfate. On evaporation of the solvent, pure salicyl alcohol was obtained, which was used in further reactions without purification.

Thionyl chloride (1.19 g, 10mmol) was slowly added to a solution of salicyl alcohol (1.24 g, 10 mmol) in dichloromethane at room temperature. After the addition of thionyl chloride the

reaction mixture was refluxed for 2 h. The reaction mixture was cooled to 0 °C and amine (10 mmol) was slowly added to it. The product was precipitated as a white powder. The product was filtered, washed with dichloromethane and kept under vacuum for 24 hr. Their chemical structures were confirmed from IR, <sup>1</sup>H and <sup>13</sup>C NMR and MS data.

#### **4-(2-hydroxybenzyl) morpholin-4-ium chloride (Compound I)**

Yield 56.0 %; mp 140 °C; IR (neat): 3391, 3048, 2944, 1604, 1432, 1130, 793; <sup>1</sup>H NMR (300 MHz, D<sub>2</sub>O): δ 7.32-6.95 (m, 4H), 4.69 (s, 2H), 3.87 (t, *J* = 5.1Hz, 4H), 3.22 (t, *J* = 5.1Hz, 4H); <sup>13</sup>C NMR (75 MHz, D<sub>2</sub>O): δ 45.7 (2C), 53.6, 66.2 (2C), 118.4, 123.1, 123.1, 134.7, 135.6, 159.3; MS (m/z): M<sup>+</sup> 193, 146, 134, 107, 86, 76. CHN analysis required for C<sub>11</sub>H<sub>16</sub>ClNO<sub>2</sub>: C, 57.52; H, 7.02; N, 6.10. Found: C, 57.32; H, 6.88; N, 5.84.

#### **1,4-bis(2-hydroxybenzyl)piperazine-1,4-diium chloride (Compound II)**

Yield 62.3 %; mp 230 °C (Decomposition); IR (neat): 3479, 3007, 2926, 2783, 1620, 1433, 1139; <sup>1</sup>H NMR (300 MHz, D<sub>2</sub>O): δ 7.29-6.91 (m, 8H), 4.69 (s, 4H), 3.49 (s, 8H); <sup>13</sup>C NMR (75 MHz, D<sub>2</sub>O): δ 43.0 (4C), 50.8 (2C), 118.4 (2C), 119.2 (2C), 123.2 (2C), 134.2 (2C), 134.9 (2C), 158.2 (2C); MS (m/z): M<sup>+</sup> 298, 278, 192, 107, 85, 56. CHN analysis required for C<sub>18</sub>H<sub>24</sub>Cl<sub>2</sub>N<sub>2</sub>O<sub>2</sub>: C, 58.23; H, 6.52; N, 7.54. Found: C, 57.97; H, 6.43; N, 7.39.

### **Pharmacology**

#### **Animals and animal husbandry**

*Sprague-Dawley* rats of either sex (150-200 g) were used in the study. The animals were bred in the Animal House and Bioassay Laboratories of the Department of Pharmacy, University of Peshawar. Animals were kept in standard cages with free access to standard laboratory food and water available *ad libitum*. Experiments were conducted between 8:00am-4:00pm, on a 12hr-12hr light dark cycle with temperature maintained at 22±2°C.

#### **Ethical approval**

The study was approved under the project entitled “Evaluation of the anti-inflammatory, antinociceptive and antipyretic activities of selected synthetic and natural compounds in animal models” by the “Ethical Committee of Department of Pharmacy, University of Peshawar, Peshawar, Khyber Pakhtunkhwa (KP), Pakistan” in its 3<sup>rd</sup> meeting held on June 15, 2011 and an approval certificate bearing number 15/EC/Pharm was obtained.

### **Evaluation of ulcerogenicity of aspirin, compound I (4-(2-hydroxybenzyl) morpholin-4-ium chloride), compound II (1, 4-bis (2-hydroxybenzyl) piperazine-1,4-diium chloride )**

Evaluation of the ulcerogenic effects of aspirin, compound I and II was carried out according to a method reported previously (Virupaksha and Shivkumar, 2008). Briefly, the animals were randomly divided into four treatment groups (n=6 each). Treatments, doses and the route of administration are summarized in Table 2.

### **Drug administration and surgical procedure**

Test compounds and aspirin in their respective doses, were dissolved in normal saline vehicle and administered orally (p.o.) by feeding tube once a day for six days. The final dose for each group was administered to 36-hour fasted animals 1 hour prior to a pyloric ligation procedure (Mabrouk et al., 2009) under ketamine (i.p.) anesthesia. Keeping in view the relationship between an empty stomach, increased acidity and the propensity for ulcerogenicity, we opted for a 36-hour fasting period (extended duration). The majority of NSAIDs are readily absorbed from the gastrointestinal tract (GIT) within 60 minutes of administration to produce systemic effects. However, there is a relatively prolonged gut transit time after oral administration increasing the possibility of localized effects on the gastric mucosa after 60 min. Consequently, we have selected a one-hour pretreatment period for the test compounds prior to the ligation procedure.

During the surgical procedure, the stomach was carefully exposed and the pyloric sphincter was immediately ligated with surgical silk avoiding any injury to blood vessels and the abdominal



wall was subsequently reclosed with sutures prior to recovery. Food and water were withheld for 4 hours to permit gastric juice to accumulate and after killing, the stomachs were re-exposed. A knot was applied at the esophageal end to avoid drainage of gastric juice and samples of the stomach tissue were dissected for histological studies.

### **Macroscopic assessment of stomach mucosa**

After ligation for 4 hrs, each stomach was removed and opened along the greater curvature to assess the degree of ulceration in terms of ulcer score under a magnifying glass (10X). Ulcer scoring was performed according to a previously reported method (Patidar, 2011) as depicted in Table 3.

### **Biochemical gastric juice analysis**

The volume of collected gastric juice was measured then; it was centrifuged at 1000 rpm for 10 minutes at room temperature and the supernatant separated for pH, free acidity, total acidity and pepsin determination. Gastric parietal cells produce hydrochloric acid (HCl), which can exist in the gastric juice either as free HCl representing free acidity and HCl bound to other acidic compounds including acidic proteins in the gastric juice (bound acidity). The term total acidity is used to designate the sum of the free acidity, bound acidity and acidity due to other weak acids (organic acids and proteins).

Two to three drops of Topfer's reagent (dimethylamino-azobenzene) were added to 1mL of supernatant and titrated against 0.01N NaOH the end point being a color change from light red to yellowish orange. The volume (ml) of alkali required represents free acidity and was determined by the following formula (Patidar, 2011; Reddy et al., 2012).

$$\text{Acidity} = \text{Volume of NaOH} \times N \times 100 \text{ mEq/L}/0.1$$

where N= normality of NaOH

Total acidity was determined by adding phenolphthalein indicator and titration with 0.01N NaOH continued until a red color reappeared. The volume of alkali consumed was substituted in the acidity equation above (Anoop and Jegadeesan, 2003; Kore Kakasaheb et al., 2011; Muniappan and Sundararaj, 2003).

To determine pepsin activity, gastric juice (0.1 ml) was mixed with 1 ml of 0.5% of bovine serum albumin (BSA) in 0.01N HCl. The mixture was then incubated at 37 °C for 20 minutes. A gastric juice blank containing no albumin was prepared by adding 1ml of 0.01N HCl. After incubation, the hydrolysis reaction was halted using 2 mL of 10 % TCA (trichloroacetic acid). All tubes were heated in a boiling water bath for 5 minutes which caused protein denaturation, it was then cooled and the precipitate was removed by centrifugation (9000 xg, 10 minutes). To 1 ml of supernatant sample of each tube 0.4 ml NaOH (2.5N) plus 0.1ml Folin-Ciocalteau phenol reagent was added. Lastly, volume was adjusted with distilled water up to 10 mL and protein absorbency was measured with ultraviolet visible (UV/VIS) spectrophotometer at 700 nm wavelength. Activity was expressed in terms of micro moles of tyrosine/ml or mg/dl bovine serum albumin (Kalra et al., 2011).

**Histological determination of ulcerogeneic effect of compound I [4-(2-hydroxybenzyl) morpholin-4-ium chloride], compound II [1,4-bis(2-hydroxybenzyl) piperazine-1,4-diium chloride] and aspirin.**

Gastric tissues were collected at the end of the experimental procedure and subjected to histological examination using a rotary microtome (SLEE Mainz CUT 5062, Germany) to produce 3 µm tissue sections and a light microscope (Labomed Lx 400 USA with digital camera Labomed iVu 3100). Practice procedures were employed for tissue processing and hematoxylin and eosin (H & E) staining with slight modifications (Falkeholm et al., 2001).

## **Results**

### **Effects of compound I, compound II and aspirin on biochemical parameters**

One way ANOVA followed by Dunnett's post hoc analysis revealed significant biochemical changes in gastric juice volume, pH, free acidity and total acidity but not pepsin concentration in the aspirin treated group. No biochemical changes were observed for the compound I and compound II treated groups (Figures 2a, 2b, 2c, 2d and 2e).

### **Effects of compound I, compound II and aspirin on the gross photomicrographic appearance of stomach mucosae**

Examination of photomicrographs, revealed no gross morphological changes such as spot ulcers or, hemorrhagic streaks in the stomach mucosae of animal groups treated with compounds I and II and saline. However, visually detectable changes were observed in the mucosae of the aspirin treated group (Figures 3a, 3b, 3c, 3d, 3e and 3f).

### **Effects of compound I, compound II and aspirin on ulcer score and body weight**

One way ANOVA followed by Dunnett's post hoc analysis revealed a significant increase in ulcer score ( $P < 0.001$ ) in the aspirin but not in the compound I and II treated groups (Figure 4a). No significant change in body weight was observed in all treated groups (Figure 4b).

### **Effects of compound I, compound II and aspirin on gastric histology**

Results of histological examination of control, aspirin, compound I and compound II are depicted in Fig. 5a, 5b, 5c, 5d, 5e and 5f [H & E stain; 10X]. Histological examination of gastric mucosae showed that oral administration of compound I and compound II resulted in no significant morphological changes. On the other hand, changes like disruption of epithelial lining and edema were clearly observed in the case of the aspirin treated group (Fig. 5b).

## Discussion

The synthesized salicyl alcohol nitrogen containing derivatives i.e. compound I and compound II were evaluated for ulcerogenic properties in comparison with aspirin. The finding of this study indicated that these compounds were devoid of ulcerogenic properties as there was no change in both biochemical and histological parameters at doses that have shown significant antinociceptive, anti-inflammatory and antipyretic activities (Ali et al., 2013).

There is an explicit need for a substitute to classical nonsteroidal anti-inflammatory drugs such as aspirin possessing effective anti-inflammatory, anti-nociceptive and antipyretic activities ~~yet~~ but devoid of serious adverse effects like GIT ulceration, hemorrhage and perforation (Singh and Rosen, 1998).

Utilizing FRED 2.1 and MOE software, molecular docking and dynamics analyses have shown better binding energy of these compounds with cyclooxygenase-2 (COX-2) enzyme, predicting these compounds as potential COX-2 inhibitors (Ali et al., 2013). Based on computational studies, the compounds have exhibited dose dependent antinociceptive, anti-inflammatory and antipyretic activities in animal models. Nonetheless, these compounds did not show antinociceptive properties in the hot plate test when compared with centrally acting standard analgesic (morphine), thus suggesting a peripheral mechanism of action in the mediation of antinociception (Ali et al., 2013).

Bioisosterism has unique relevance in the field of pharmaceutical sciences and is performed to curtail side effects or to alter the activity of a target molecule (Wanget al., 2003). In the biomedical field, the aim of exchanging one bioisostere for another is to boost the preferred pharmacological, biological or physical qualities of a substance without making substantial changes in the chemical skeleton (Lima and Barreiro, 2005; Patani et al., 1996).

In this study, salicyl alcohol with its nitrogen containing derivatives, that is compounds I and II, were investigated for ulcerogenic potential in comparison to aspirin. The doses of the compounds selected for this study (100 and 150 mg/kg) were based on our previous observations that these doses possessed significant antinociceptive, anti-inflammatory and antipyretic activities (Ali et al., 2013).

Results of the current study clearly demonstrated that treatment with I and II, compared to aspirin, lacked ulcerogenic potential as there was no significant change in gastric juice volume, pH, free or total acidity, pepsin concentration or ulcer score. Since these compounds possess neutral characteristics, it may be concluded that their presence in the stomach may not have influenced either the acidity or other biochemical parameters of gastric juice to any significant extent.

The gross appearance of gastric mucosa revealed an absence of morphological changes like ulcer spot or hemorrhagic streaks in the saline vehicle control treated as well as compound I and compound II treated groups in contrast to the aspirin group where such changes were clearly evident. Moreover, histological examination of the stomach tissues revealed no surface epithelial disruption, deep mucosal damage, edema, necrotic lesion or leucocytes infiltration following treatment with saline, compound I or compound II while changes like mucosal disruption and edema were visible in the group treated with aspirin. Furthermore, during the entire period of the study, no significant changes in body weight or any other sign of toxicity were observed in the compound I and compound II treated animals.

The lack of ulcerogenic properties of compound I and II may be attributed to the absence of a free carboxylic acid group (-COOH) which is generally considered an important contributor towards the ulcerative properties of the majority of NSAIDs including aspirin (Shanbhag et al., 1992). Additionally, as evidenced by our previous evaluation studies on molecular docking and

dynamics simulation, these compounds interact with COX II thus favoring non-ulcerogenic properties (Ali et al., 2013).

## **Conclusions**

In summary, the current study presents the bioisosteric synthesis and evaluates the ulcerogenic potential of the synthetic compounds (I and II) versus aspirin. Our findings clearly indicate that unlike aspirin, these compounds lack significant ulcerogenic properties that might be ascribed to selective inhibition of COX-II enzyme, the absence of a free carboxylic acid moiety and consequently, the neutral characteristics of these compounds.

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## **Conflict of Interest**

We declare that we have no conflict of interest.

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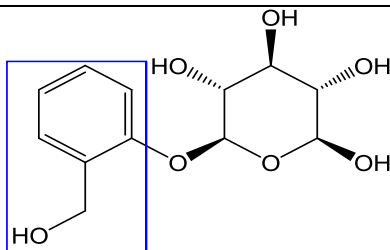
Yoon S H, Fulton DB, Robyt JF (2004) Enzymatic synthesis of two salicin analogues by reaction of salicyl alcohol with *Bacillus macerans* cyclomaltodextrin glucanyltransferase and *Leuconostoc mesenteroides* B-742CB dextranucrase. *Carbohydr Res* 339:1517-1529

**Table 1.** Chemical structures of salicin, salicyl alcohol, compound I, II and acetyl salicylic acid.

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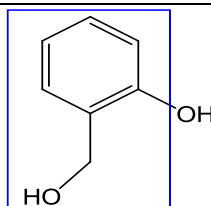
Chemical structures

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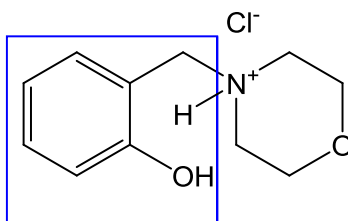
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Salicin



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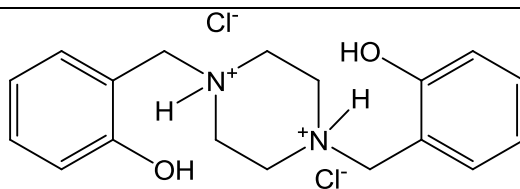
Salicyl alcohol



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Compound (I)

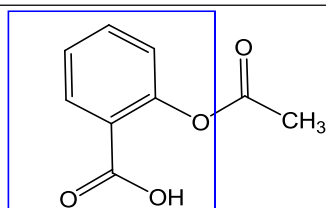
[4-(2-hydroxybenzyl) morpholin-4-ium chloride]



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Compound (II)

[1,4-bis(2-hydroxybenzyl)piperazine-1,4-dium chloride]



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Acetyl salicylic acid

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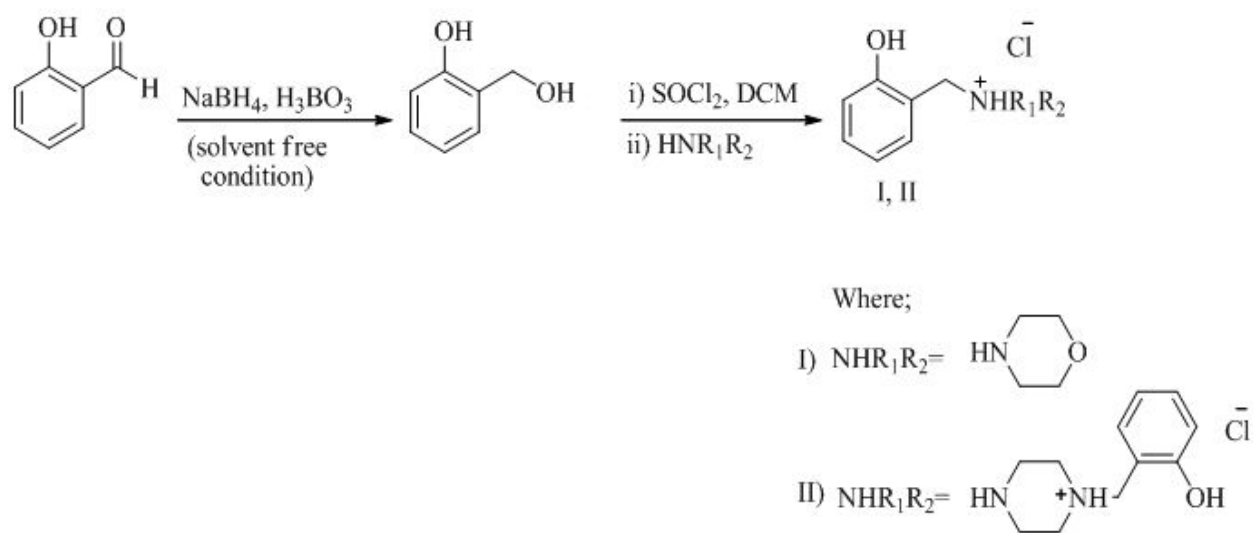
**Table 2.** Distribution of animals in treatment groups.

Group No.	Treatment group	Dose ( mg/kg)	Route (per os)
I	Saline control (untreated)	-	p.o
II	Standard treatment group (Aspirin)	150	p.o
III	Test treatment group (compound I)	100	p.o
		150	
IV	Test treatment group (compound II)	100	p.o
		150	

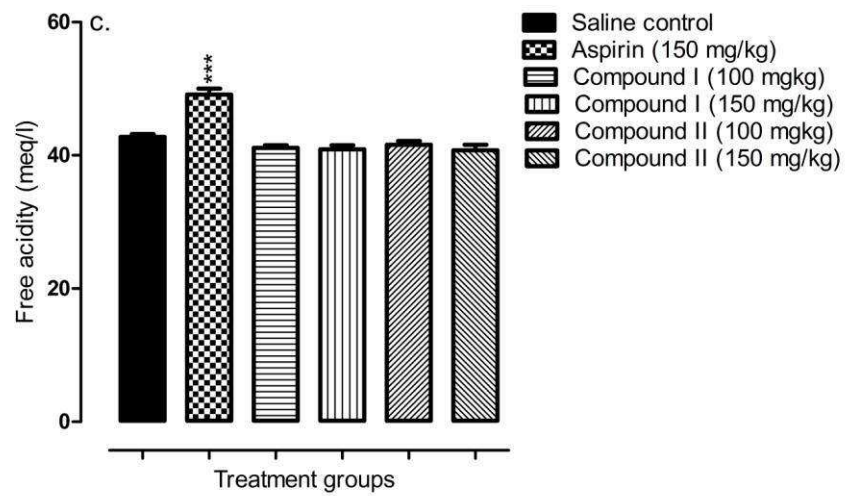
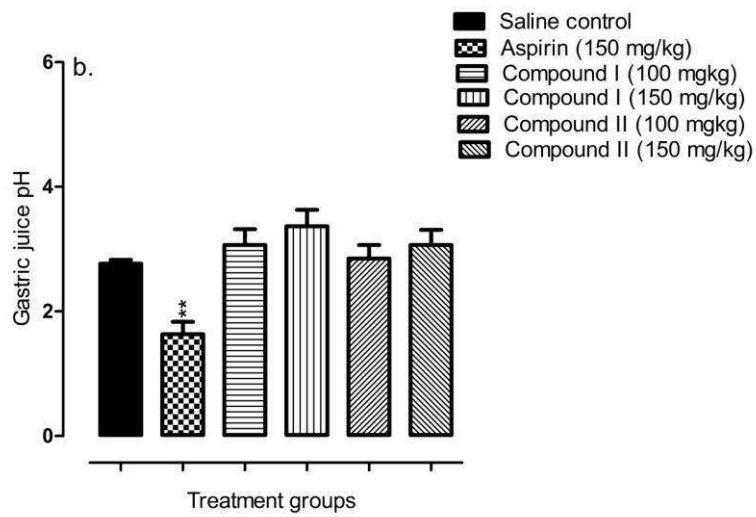
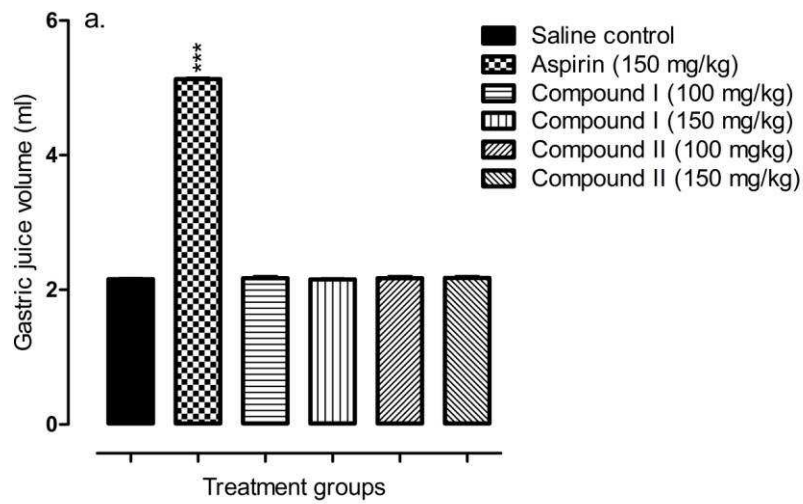
**Table 3.** Determination of ulcer score.

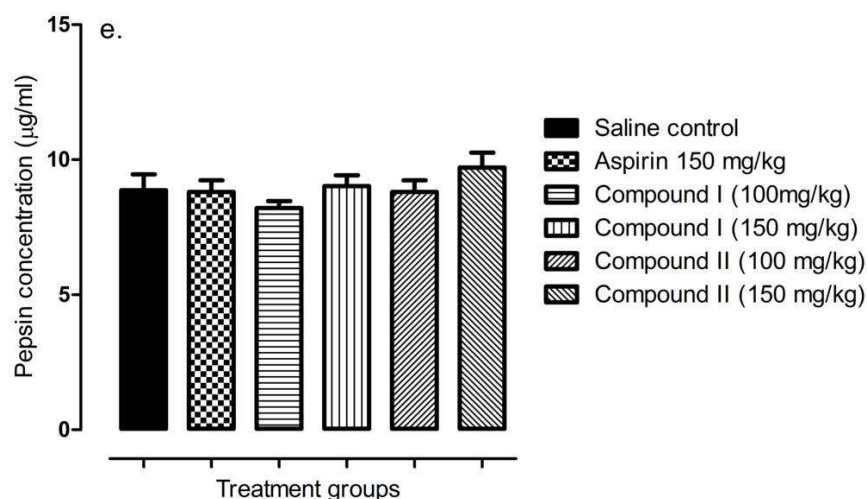
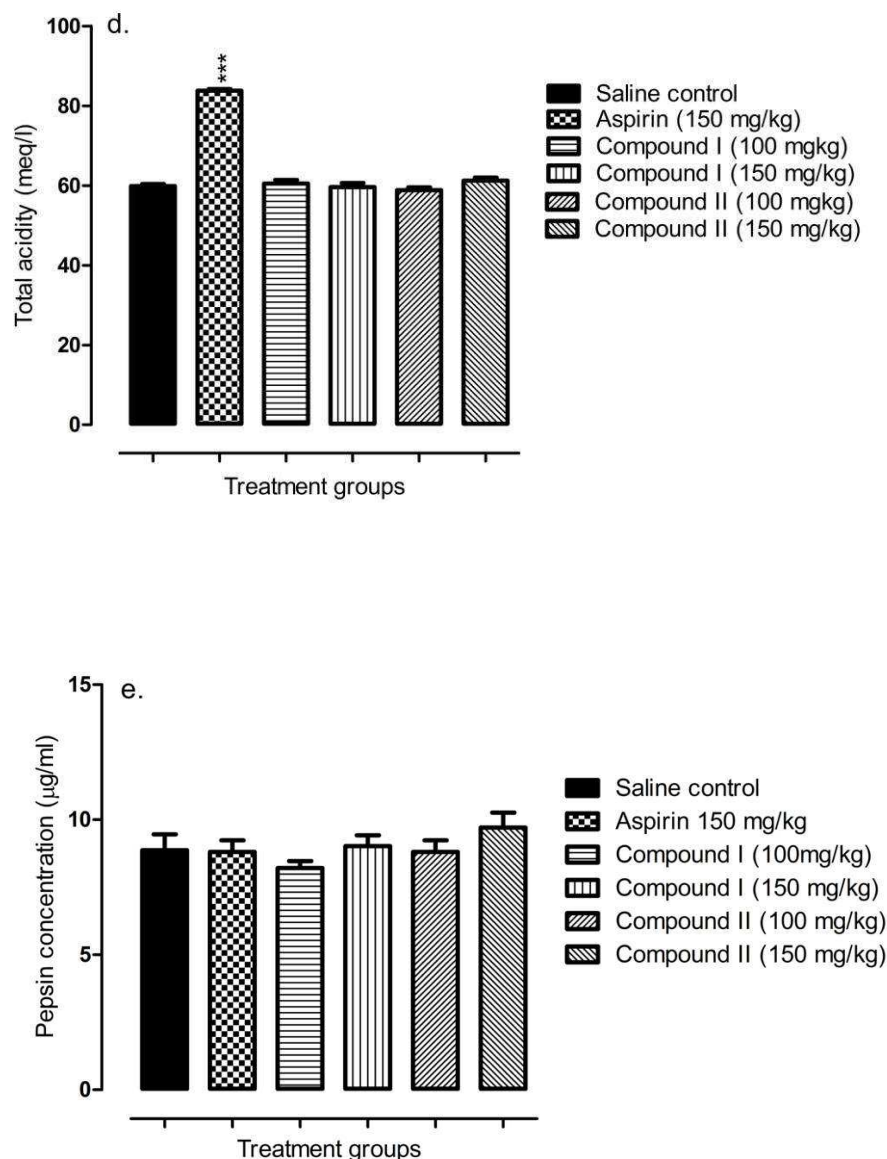
Score	Observation
0	Normal stomach
0.5	Red coloration
1.0	Spot ulcers
1.5	Hemorrhagic streaks
2.0	Ulcer number $\geq 3$ but $\leq 5$
3.0	Ulcer number $> 5$

Mean ulcer score for each animal group was calculated.

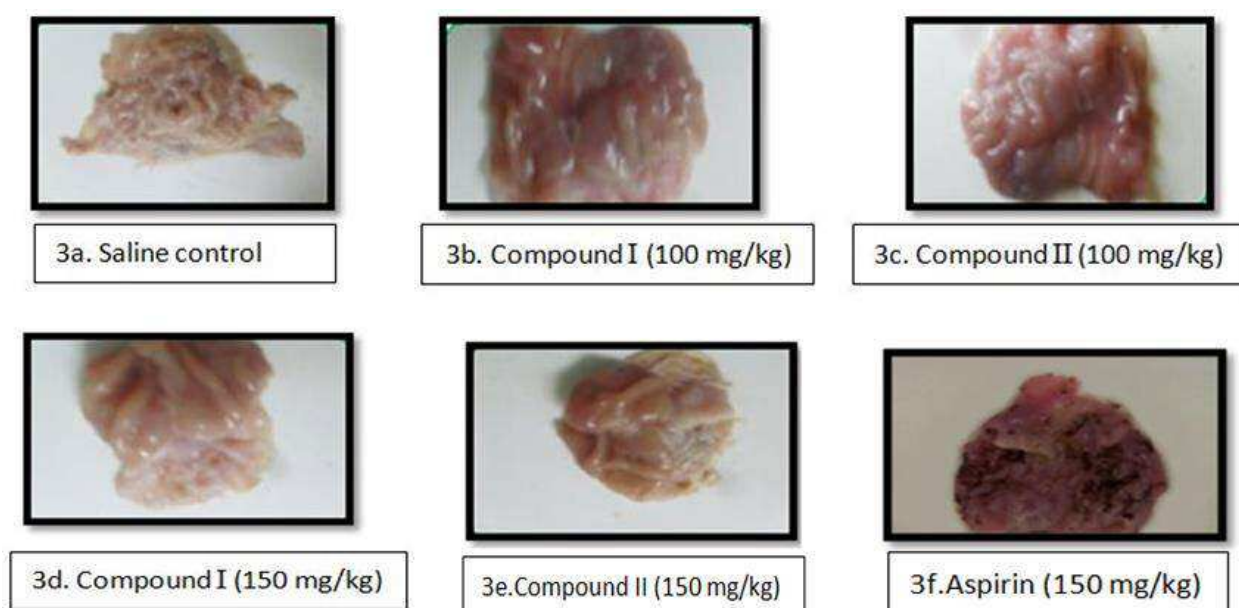


**Figure 1.** Synthesis of compound I [4-(2-hydroxybenzyl)morpholin-4-ium chloride] and compound II [1,4-bis(2-hydroxybenzyl)piperazine-1,4-diium chloride].



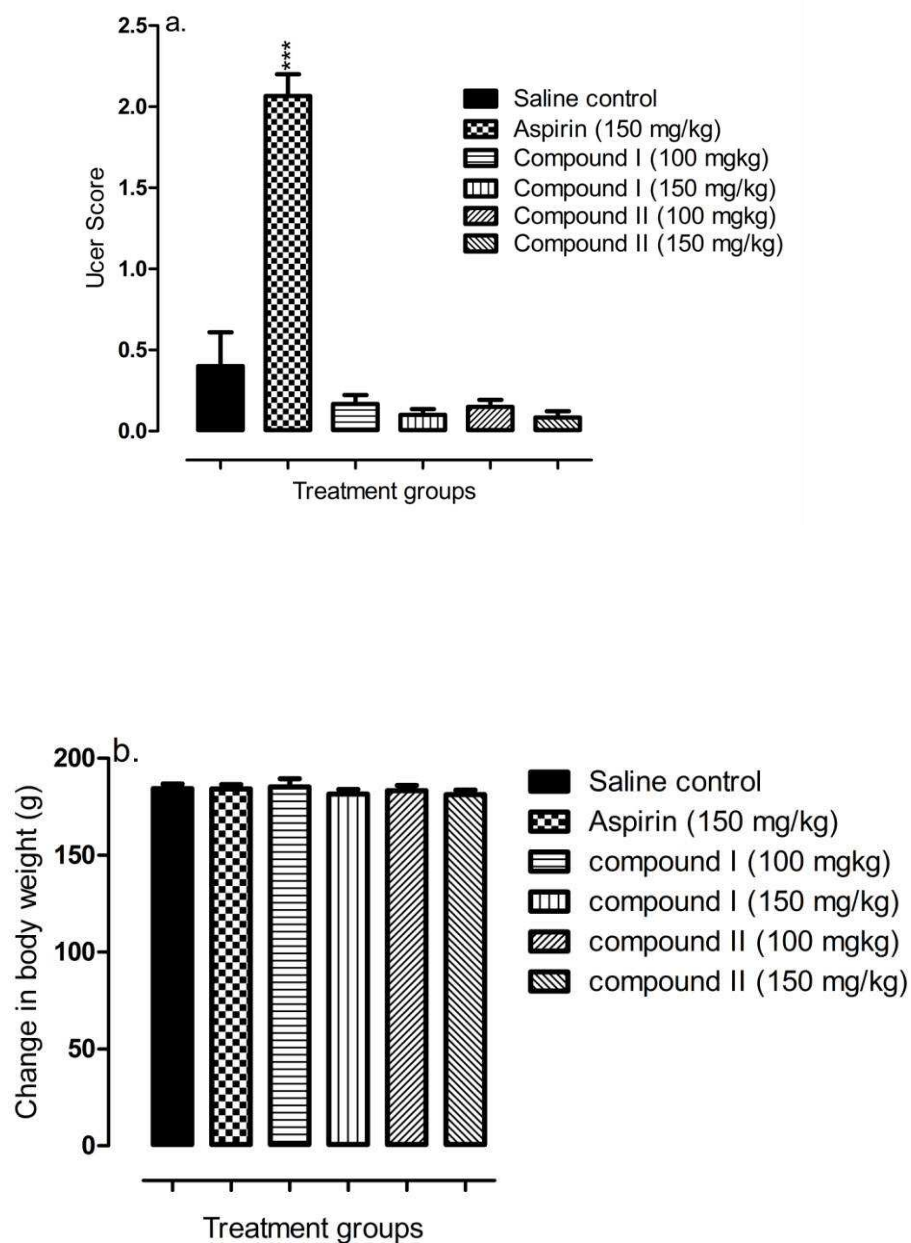


**Figure 2.** Effect of aspirin, compound I, II on gastric juice volume, pH, free acidity, total acidity and pepsin concentration. Saline (control), aspirin (150 mg/kg), compounds I and II (100 and 150 mg/kg) were administered once a day orally for six days. The final dose was administered to 36-hour fasted rats in each experimental group 1 hr before pyloric ligation. Four hrs after surgery, gastric juice was collected and analyzed for volume (Figure 2a), pH (Figure 2b), free acidity (Figure 2c), total acidity (Figure 2d) and pepsin concentration (Figure 2e). Data presented as mean  $\pm$  S.E.M. (n=6) compared to the saline vehicle treated group \*\*P<0.01, \*\*\*P<0.001 (One way ANOVA followed by Dunnett's post-hoc analysis).

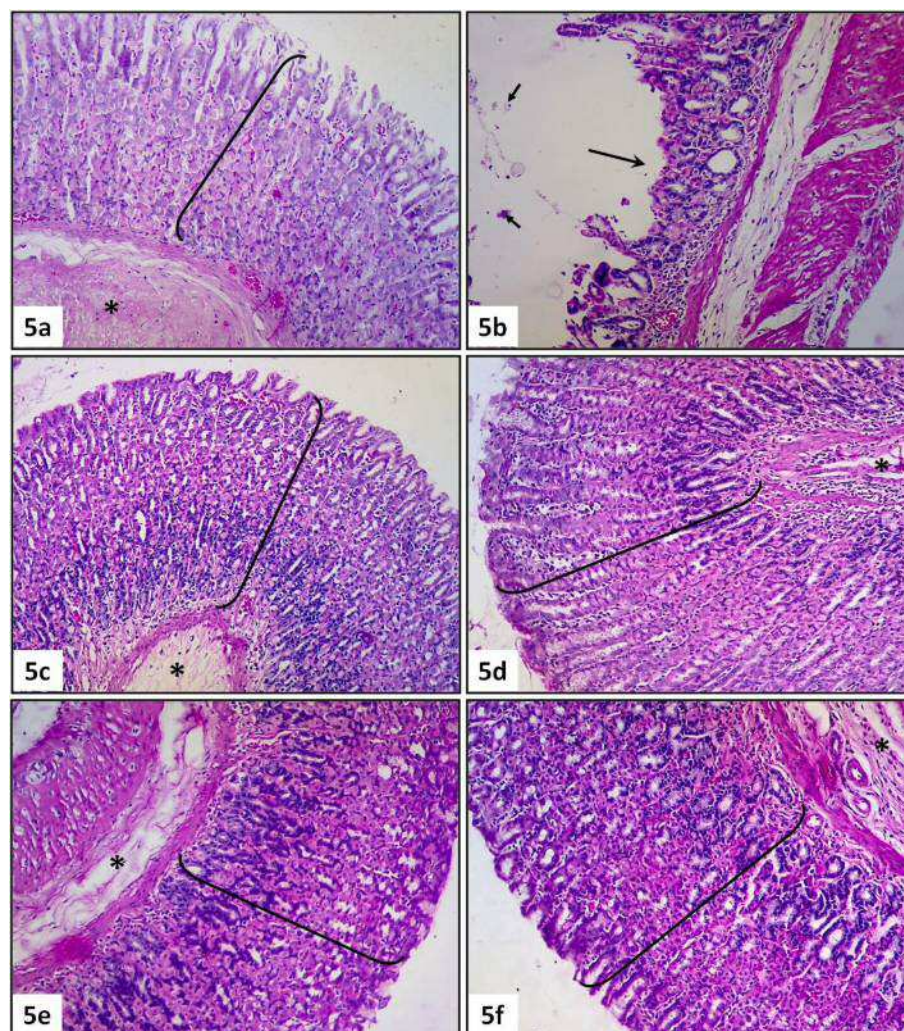


**Figure 3.** Gross photomicrographic appearance of the gastric mucosae of saline (control) (Figure 3a), compound I (100mg/kg, Figure 3b; 150 mg/kg, Figure 3d), compound II (100 mg/kg, Figure 3c; 150 mg/kg; Figure 3e) and aspirin (150 mg/kg; Figure 3f) treated groups. For each condition (repeated 6 times) a representative photomicrograph is shown (n=6).





**Figure 4.** Effect of aspirin, compound I, II on ulcer score and body weight. A significant increase in ulcer score was observed for the aspirin but not for the compound I and II treated groups (Figure 4a). There was no significant change in body weight of any treated group (Figure 4b) (n=6 each). \*\*\*P<0.001 (One way ANOVA followed by Dunnett's post-hoc analysis).



**Figure 5.** Histological ulcerogenic effects of control, aspirin, compound I or compound II (H & E staining; 10x original magnification) (n=6 each). Representative photomicrographs of the glandular portion of the stomach: (a) the saline (control group) showing normal appearance of mucosa (bar) and submucosa (asterisk), (b) aspirin treated group (150 mg/kg) showing erosion with disruption of mucosa (large arrow) and inflammatory exudates (small arrow). Normal histological appearance of mucosa (bar) and sub mucosa (asterisk) were found in groups of animals treated with: (c) compound I (100 mg/kg), (d) compound I (150 mg/kg), (e) compound II (100 mg/kg) and (f) compound II (150 mg/kg).